

# Connexin expression in electrically coupled postnatal rat brain neurons

Laurent Venance<sup>\*†</sup>, Andrei Rozov<sup>‡</sup>, Maria Blatow<sup>\*</sup>, Nail Burnashev<sup>‡</sup>, Dirk Feldmeyer<sup>‡</sup>, and Hannah Monyer<sup>\*§</sup>

<sup>\*</sup>Department of Clinical Neurobiology, University Hospital for Neurology, Im Neuenheimer Feld 364, and <sup>‡</sup>Max-Planck-Institut für Medizinische Forschung, Jahnstrasse 29, D-69120 Heidelberg, Germany

Edited by Michael V. L. Bennett, Albert Einstein College of Medicine, Bronx, NY, and approved June 16, 2000 (received for review January 28, 2000)

**Electrical coupling by gap junctions is an important form of cell-to-cell communication in early brain development. Whereas glial cells remain electrically coupled at postnatal stages, adult vertebrate neurons were thought to communicate mainly via chemical synapses. There is now accumulating evidence that in certain neuronal cell populations the capacity for electrical signaling by gap junction channels is still present in the adult. Here we identified electrically coupled pairs of neurons between postnatal days 12 and 18 in rat visual cortex, somatosensory cortex, and hippocampus. Notably, coupling was found both between pairs of inhibitory neurons and between inhibitory and excitatory neurons. Molecular analysis by single-cell reverse transcription-PCR revealed a differential expression pattern of connexins in these identified neurons.**

In the brain, gap junctions represent an important means of intercellular communication. Connexins (Cx) are the gap junction-forming proteins of vertebrates, and the multigene family comprises at least 14 different Cx (1–3). Of the seven Cx known to be present in the brain, Cx26, Cx32, Cx43 (4), and the recently discovered Cx36 (5, 6) are the most abundantly expressed.

Intercellular communication via gap junctions plays an important role in the developing nervous system before chemically mediated synaptic transmission becomes the major mode of neuronal communication. Thus, extensive dye coupling during early corticogenesis has indicated the presence of gap junctions between clusters of neuroblasts in the ventricular zone (7). Coupling during early postnatal development was further documented by Ca<sup>2+</sup> imaging in slices (8, 9) and by disruption of dye coupling by gap junction blockers (10). The expression analysis of Cx26, Cx32, and Cx43 revealed their differential localization and their unique temporal expression profiles, which correlate with important developmental events such as cell proliferation, migration, and neuronal circuit formation (4, 11).

It was thought that electrical synapses play a subordinate role in the adult brain, compared with what appeared to be the preponderant way of intercellular communication, the chemical synapse. However, there is a growing body of morphological and electrophysiological evidence indicating that electrical synapses are also present in the juvenile and adult brain. Two recent studies deserve particular attention because they indicate that in addition to some brain structures that were known to contain extensive neuronal coupling by gap junctions (inferior olive, retina, neurons of the olfactory bulb, locus ceruleus; ref. 12), cortical neurons may also use this way of cellular communication quite commonly (13, 14). In both studies, electrical synapses were found between cortical inhibitory interneurons.

The functional significance of neuronal gap junctions in the adult brain has remained elusive. In addition to mediating the exchange of metabolic factors, gap junctions may provide for temporal coordination of neuronal activity (15–18). Of note are *in vitro* studies (19) that provide evidence for the role of gap junctions in the generation of high-frequency oscillations. Furthermore, *in vivo* studies (20–22) also suggest that gap junctions may play a role in the generation of oscillatory activity.

Compelling evidence for a role for neuronal gap junctions in the mature brain is indicated by the persistent expression of Cx36 in many structures in the adult brain (5, 6, 23). However, it is not known which neuronal cell types express this Cx or whether a given type of neuron expresses more than one Cx.

In this paper we present functional and molecular evidence that pairs of neurons in the visual cortex, somatosensory cortex, and hippocampus of juvenile rats are electrically coupled. The characterization of the electrically coupled neurons is based on morphological, electrophysiological, and immunocytochemical properties. The distinct expression profiles of Cx in these neurons were analyzed by single-cell reverse transcription-PCR (RT-PCR).

## Materials and Methods

**Electrophysiology.** The preparation of brain slices from rat [postnatal days (P12–P18)] has been described elsewhere (24–26). During recording and intracellular labeling, slices were maintained at 34–36°C and continuously superfused with an extracellular solution containing (in mM) 125 NaCl, 2.5 KCl, 25 glucose, 25 NaHCO<sub>3</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 2 CaCl<sub>2</sub>, and 1 MgCl<sub>2</sub>, bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>. The pipette (intracellular) solution contained (in mM) 105 potassium gluconate, 30 KCl, 10 Hepes, 10 phosphocreatine, 4 ATP-Mg, and 0.3 GTP (adjusted to pH 7.3 with KOH). Biocytin (2–5 mg/ml; Sigma) was routinely added to the internal solution.

For electrophysiological recordings slices were placed in the recording chamber under an upright microscope (Axioskop; Zeiss) fitted with 2.5× plan/0.075 numerical aperture and 40×-W/0.80 objectives. Individual neurons were identified at 40× magnification, using infrared-differential interference contrast (IR-DIC) microscopy (27, 28). All cell types used in this study were readily identifiable by their shape and action potential firing pattern (26, 29, 30).

Electrically coupled cells were about 10–40 μm apart. After we established the whole-cell mode, using patch pipettes with a resistance of 4–8 MΩ, depolarizing and hyperpolarizing current pulses were applied to one of the potentially coupled neurons. Voltage responses recorded in current-clamp mode from the other cell indicated electrical coupling between these neurons. The success rate of finding an electrical coupling was calculated as the inverse (in %) of the number of neurons tested before gap junction coupling was found. Thus, for instance, for electrically

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: Cx, connexin(s); RT-PCR, reverse transcription-PCR; Pn, postnatal day *n*; IR-DIC, infrared-differential interference contrast; GABA,  $\gamma$ -aminobutyrate.

<sup>†</sup>Present address: Laboratoire de Neuropharmacologie INSERM U-114, Collège de France, 11 Place M. Berthelot, 75005 Paris, France.

<sup>§</sup>To whom reprint requests should be addressed at: Max-Planck-Institut für Medizinische Forschung, Jahnstrasse 29, D-69120 Heidelberg, Germany. E-mail: monyer@mpimf-heidelberg.mpg.de.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.160037097. Article and publication date are at [www.pnas.org/cgi/doi/10.1073/pnas.160037097](http://www.pnas.org/cgi/doi/10.1073/pnas.160037097)

coupled pairs of bipolar interneurons, first a neuron with the appropriate appearance and action potential firing pattern was identified. Then we counted the number of neurons with the same properties that had to be patched before electrical coupling was found (the last neuron was included in the count). Hence electrical coupling between bipolar interneurons was found after one or two tries, whereas for electrical coupling between a fusiform interneuron and a spiny stellate cell about 16 cells had to be tested on average. The coupling coefficient between two cells was determined as the ratio of the voltage response in cell 2 divided by the voltage response in cell 1 under steady-state conditions. For all types of neuronal pairs that were studied here, examples could be found where only chemical synapses were present. These pairs were not considered further in this study.

Recordings were filtered at 2–5 kHz and digitized at 5–10 kHz, using an ITC-16 interface (Instrutech, Mineola, NY) and the program PULSE (version 8.21; HEKA Electronics, Lambrecht, Germany), and stored on the hard disk of a Macintosh computer for off-line analysis (Igor; WaveMetrics, Lake Oswego, OR).

**Morphology.** After recording, slices were fixed at 4°C for at least 24 h in 100 mM PBS (pH 7.4) containing 4% paraformaldehyde and then processed according to a protocol described previously (31). In brief, after incubation in avidin-biotinylated horseradish peroxidase (ABC-Elite; Camon, Wiesbaden, Germany), slices were reacted, using 3,3-diaminobenzidine as a chromogen, under visual control until dendritic and axonal arbors were clearly visible. Slices were then mounted on slides, embedded in Mowiol (Hoechst Pharmaceuticals), and coverslipped. Biocytin-labeled neurons were reconstructed with NEUROLUCIDA software (Microbrightfield, Colchester, VT), using a Zeiss Axioplan microscope at a magnification of  $\times 400$  or  $\times 1000$ .

**Biocytin Filling and *in Situ* Hybridization.** For neurochemical characterization of the neurons, cells were filled with biocytin (1–4 mg/ml) dissolved in an internal pipette solution. Subsequently, the slices were fixed overnight in 4% paraformaldehyde at 4°C. Fixed slices were embedded in 4% agar and resliced at 50  $\mu\text{m}$  with a Vibratome. The biocytin-filled cells were visualized by FITC-conjugated avidin (3  $\mu\text{g}/\text{ml}$ ; Jackson ImmunoResearch). The procedures for the analysis of somatostatin and parvalbumin expression, using a nonradioactive *in situ* hybridization labeling method and antibody, respectively, are described elsewhere (32). The digoxigenin-labeled RNA probe was synthesized in a manner identical to that described by Standaert *et al.* (33).

**Single-Cell RT-PCR.** Single-cell RT-PCR was performed as previously described (34, 35). Patch pipettes used for RT-PCR experiments (1–2.5 M $\Omega$ ) were filled with autoclaved internal solution containing (in mM) 140 KCl, 3 MgCl<sub>2</sub>, 5 EGTA, and 5 Hepes (pH 7.3 adjusted with KOH). Harvesting of the cell content and expelling were performed as previously described (36). Reverse transcription was carried out for 1 h at 42°C (Superscript reverse transcriptase II; GIBCO/BRL). Subsequently, two rounds of PCR amplification were performed, with 4  $\mu\text{l}$  of the first PCR serving as a template for the second PCR. All primers were tested on plasmid and total brain cDNA, and it was verified that all Cx that are known so far to be expressed in the brain can be detected with the combinations that were used. The concentration of the primers for all amplification reactions was 0.2  $\mu\text{M}$ , except for the degenerate primers, which were 0.4  $\mu\text{M}$ . The cycling conditions were a hot start at 94°C for 5 min, 35 cycles (94°C for 30 sec, 53°C for 30 sec, 72°C for 40 sec), and an elongation step at 72°C for 10 min. Contamination artifacts were excluded both for contamination of the PCR and for inadvertent harvesting from surrounding material in the slice preparation for each experiment as previously described (36). The success rate of amplification was 50% or less (see Table 1).

We do not conclude that the other neurons do not express Cx. The most likely explanation for what we think are “false negatives” could be very low expression, insufficient amounts of harvested material, and/or loss of the harvested material during the expelling procedure. In our previous work, in which the significantly more highly expressed glutamate receptor composition was studied, the success rate of amplification was 50% in interneurons and 90% in large pyramidal neurons, yet there was functional evidence for receptor expression in all neurons.

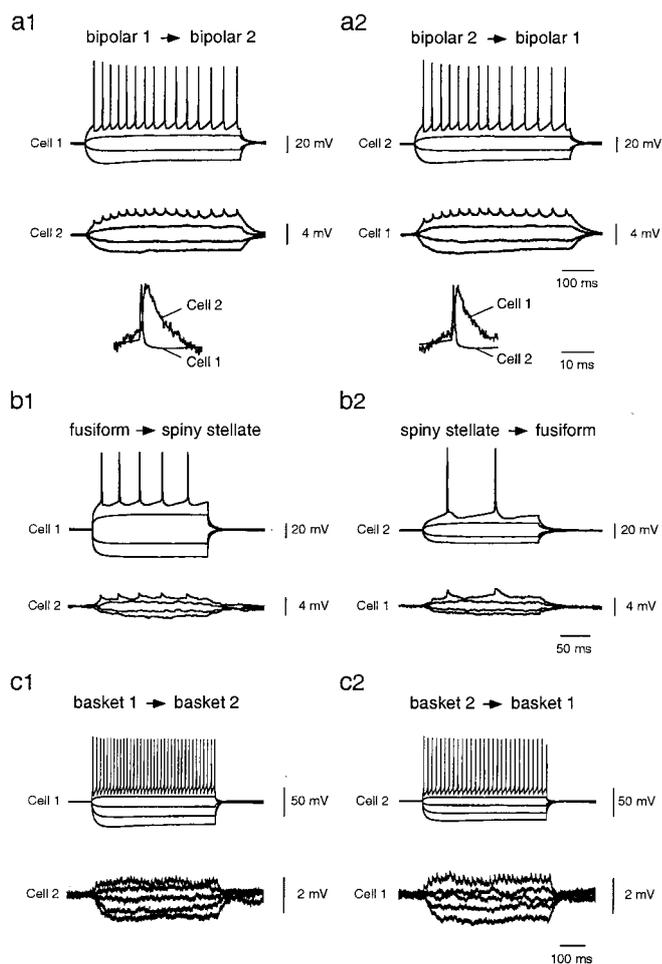
For the PCR with degenerate primers the following primers were used: cP5' 5'-GGCTGT(G/A)A(C/A/G)AA(T/C)GTCTGCTA(T/C)GAC-3' and cP3' 5'-TGGG(C/A/G)C(T/G)GGA(C/A/G)A(C/T/G)GAAGCAGT-3' for the first reaction. For the second reaction the same 3' primer and the nested cP5'n 5'-TTCCCCATCTC(C/T/G)CA(C/T)(G/A)T(C/T/G)CG-3' were used. For the Southern analysis of the amplicons obtained with the degenerate common primers, the following oligonucleotide probes were used: 5'-CATGATGTA-AAAGACATACATGAAGACAGCTTCGAAGATGAC-CCG-3' for Cx26, 5'-CTTTACCTCTCCAGGTGAAGG-GGGTCCCCATGCCC-3' for Cx32, and 5'-ATCTCCACGTT-GACCCGTCGGTCTGCGCCACTTTG-3' for Cx43. For specific amplification of Cx26 the following primers were used: P5' 5'-ACTCCGGACCTGCTCCTTAC-3' and P3' 5'-CGGA(C/T)GTG(G/A)GAGATGGGGAA-3' for the first amplification. The second amplification was performed with the same 5' primer and the nested P3'n 5'-GTCGTAGCACACATTCTTACAGCC-3'. The probe for the Southern blot was 5'-GTTTGT-TGACACCCCCGAGGATGCTCTGGAGTGTGCC-3'.

The specific amplification of Cx32 was carried out with the following primers: P5' 5'-CCCTACACAGACATGAGACC-3' and P3' 5'-CGGA(C/T)GTG(G/A)GAGATGGGGAA-3' primer for the first amplification. The same 5' primer and the nested P3'n 5'-GTCATAGCAGACGCTGTTACAGCC-3' were used for the second amplification. The probe for the Southern blot was 5'-TGCCGATTTCAGCCACTGAGCAAG-GTGTATAGACC-3'. For the specific amplification of Cx43 the following primers were used: P5' 5'-CGCTTTTACGAGGTAT-CAGCAC-3' and P3' 5'-CG(C/A/G)A(T/C)(A/G)TG(C/A/G)GAGATGGGGAA-3' for the first PCR and the same 5' primer and P3'n 5'-GTC(G/A)TAGCAGAC(G/A)TT(C/T/G)T(T/C)ACAGCC-3' for the second PCR. The specific Cx36 amplification was performed using the primers P5' 5'-ATGGGGGAATGGACCATCTTG-3' and P3' 5'-GACAGTCGAGTACCGGCGTTCTC-3' for the first amplification, and the same 5' and the nested P3'n 5'-AACAGAGACT-GGGGGTGCACACC-3' for the second amplification. The probe for the Southern blot was 5'-ATCGTACACCGTCTC-CCCTACAATGGCCAC-3'. The washing conditions were 0.5 $\times$  standard saline citrate (SSC) first at room temperature and then at 56°C.

## Results

Gap junction coupling was studied in three different neuron pairs: between bipolar  $\gamma$ -aminobutyrate (GABA)ergic interneurons in layer 2/3 of visual cortex, between fusiform GABAergic interneurons and spiny stellate cells in layer 4 of somatosensory cortex, and between hippocampal dentate gyrus basket cells.

Fig. 1*a* shows whole-cell recordings from electrically coupled bipolar GABAergic interneurons. The voltage responses in the first bipolar interneuron in response to current injection were reflected in the second cell and vice versa. However, the amplitude of the action potentials was reduced by a factor of  $\approx 50$ –100, and the shape of the action potentials was distorted in the coupled neuron, reflecting low-pass filtering of the voltage response; the coupling coefficient measured under steady-state conditions was  $0.086 \pm 0.082$  (mean  $\pm$  SD;  $n = 6$  pairs). The



**Fig. 1.** Electrical coupling between cell pairs in different regions of the postnatal rat brain. (a) Electrical coupling between two bipolar GABAergic interneurons of layer 2/3 visual cortex. (b) Electrical coupling between layer 4 fusiform interneuron and spiny stellate neuron. (c) Electrical coupling between two hippocampal dentate gyrus basket cells. In all cases, the voltage response of cell 1 after current injection is also detectable in cell 2 and vice versa, although with a significantly reduced amplitude. Fast signals like action potentials showed a clearly distorted time course. This is most visible in the bottom traces of *a*, where a single reflected voltage response in cell 2 was scaled to the amplitude of the action potential in the current-injected cell 1 and vice versa. The recordings shown here were made in the presence of 5  $\mu$ M 2,3-dihydroxy-6-nitro-7-sulfamoylbenzof[*f*]quinoxaline (NBQX) and 10  $\mu$ M bicuculline to block chemical synapses.

voltage responses transmitted via the gap junctions showed no rectification in either cell with a change in the direction of the injected currents. For pairs of bipolar neurons the success rate was  $67\% \pm 33\%$  ( $n = 11$  pairs). In only 1 of 11 pairs electrical coupling coexisted with a unidirectional inhibitory chemical synapse.

In the somatosensory cortex electrical coupling was also found at this postnatal stage of development, both between pairs of fusiform interneurons (data not shown) and between pairs of excitatory spiny stellate cells and fusiform interneurons, whereas electrical coupling between pairs of spiny stellate cells was not observed. A recording from an electrically coupled pair of a fusiform interneuron and a spiny stellate cell is shown in Fig. 1*b*. The mean coupling coefficient for this type of cell pair was  $0.05 \pm 0.038$  ( $n = 11$  pairs); the success rate of finding a connection was  $6.4\% \pm 4.3\%$ . We did not find coupling via

chemical synapses in any pairs where electrical coupling was found.

Finally, the presence of electrical coupling between pairs of hippocampal dentate gyrus basket cells was investigated, because a previous anatomical study had indicated the presence of gap junctions in this cell population (37). Electrical coupling between a pair of basket cells is depicted in Fig. 1*c* and was found to be extremely frequent (with a success rate of  $82\% \pm 25\%$ ;  $n = 11$  pairs). Similar to voltage pulses between neocortical pairs of neurons, the voltage pulses between pairs of hippocampal dentate gyrus basket cells were transmitted in a strongly attenuated fashion from one cell to another (coupling coefficient of  $0.026 \pm 0.018$ ;  $n = 11$  pairs). Coexistence of electrical and inhibitory chemical synapses was found in 3 of 11 basket cell pairs; of these, two were reciprocal.

The identification of the electrically coupled cell types was based on their morphological, functional, and biochemical signature. The IR-DIC image and the action potential firing pattern of the bipolar interneurons in layer 2/3 of visual cortex were similar to those described previously (30). These neurons have a prominent long dendrite extending toward layer 6, with few or no oblique dendrites. The axon displays a high degree of collateralization within layers 2/3 and 1 and a strong, vertically oriented, descending projection terminating in layer 5 (38, 39) (Fig. 2*a*).

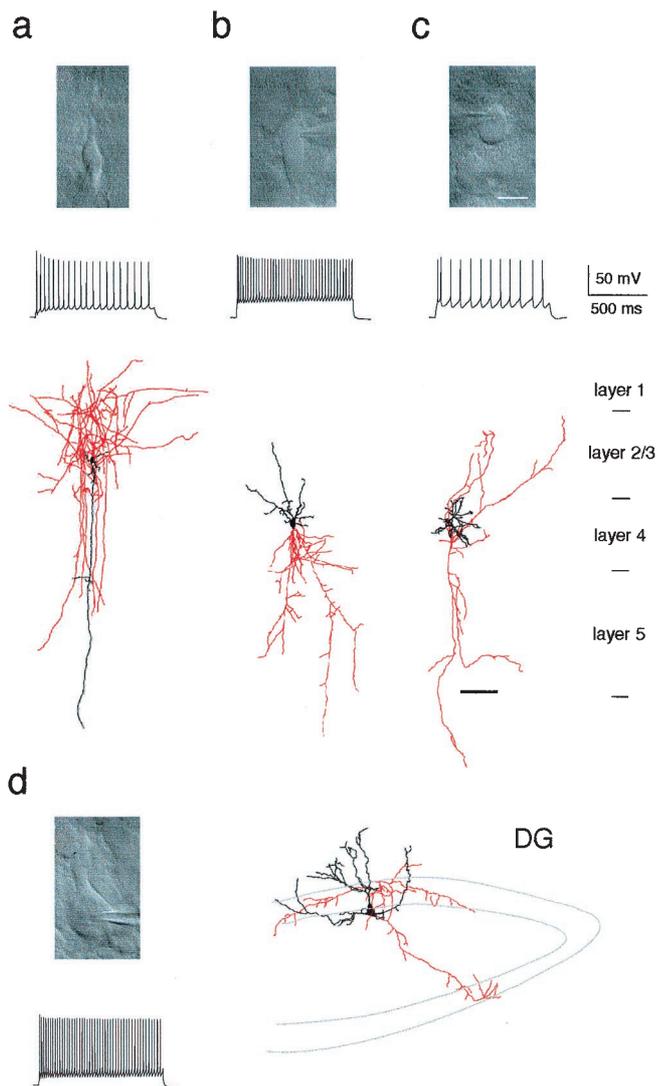
Interneurons in layer 4 of rat barrel cortex are highly diverse, as judged from their different morphologies and spiking patterns. The fusiform interneurons studied here displayed high-frequency action potential firing ( $>50$  Hz) with little spike adaptation when injected with strong depolarizing currents. Fusiform layer 4 interneurons have varicose, sparsely spinous dendrites oriented toward layer 2/3. Their axons show a dense projection within layer 4 and descending collaterals in layers 5 and 6 (Fig. 2*b*).

Spiny stellate cells of the barrel cortex display a characteristic spiking pattern with a shorter interval between the first and second spikes and a uniform, longer interval between later spikes (26). Their dendrites that are confined to layer 4 typically show an asymmetrical morphology. The axons display columnar organization with a few side branches and show dense projections within layers 4 and 2/3 and a few collaterals in lower layer 5 and upper layer 6 (26, 40, 41) (Fig. 2*c*).

Hippocampal basket cells showed the high-frequency action potential pattern and the morphological characteristics as previously described (29), namely, large-sized somata that were localized at the border of the granule cell layer and hilar region; a prominent apical dendrite projecting into the granule cell layer; basal dendrites that, after a very short extension into the hilar region, turn and project into the granule cell layer and the molecular layer; and an axon projecting into the granule cell layer (Fig. 2*d*).

The bipolar interneurons that were found to be electrically coupled in layer 2/3 of the visual cortex had previously been shown to be immunopositive for somatostatin but not for parvalbumin (30). This result was confirmed in our study, using a somatostatin-specific riboprobe (Fig. 3*a*). Of five cells that exhibited the above-described IR-DIC image and firing pattern, four were found to be somatostatin-positive. Similarly, the fusiform interneurons in the somatosensory cortex layer 4 were also somatostatin-positive (eight of eight cells) (Fig. 3*b*) and negative for parvalbumin (three of three cells; not shown). However, basket cells in the hippocampus were immunopositive for parvalbumin (seven of seven) (Fig. 3*c*).

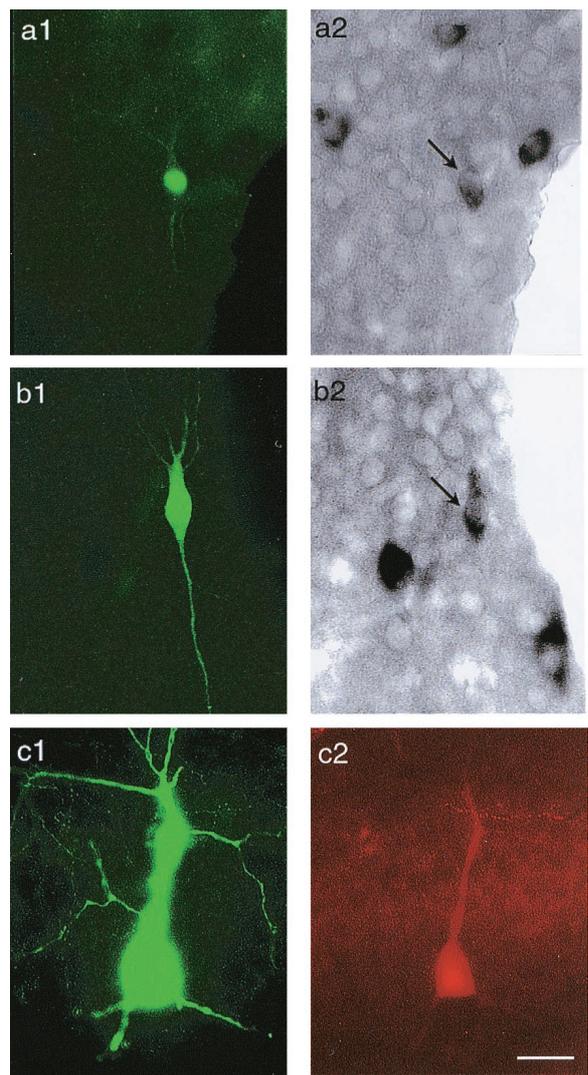
To assess the Cx expression in electrically coupled neurons, single-cell RT-PCR was performed on another set of neurons that were identified by their IR-DIC image and firing pattern. The steps involved in the Cx expression analysis are outlined in Fig. 4*a*. For each cell type, the analysis entailed separate nested



**Fig. 2.** Anatomical reconstruction and firing pattern of electrically coupled neurons. IR-DIC images, action potential firing patterns, and camera lucida-type reconstructions of biocytin-injected neurons are shown for all cell types. (a) Bipolar interneuron in layer 2/3 of P14 rat visual cortex. (b and c) Fusiform interneuron (b) and spiny stellate cell (c) in layer 4 of P14 rat somatosensory cortex. (d) Hippocampal dentate gyrus basket cell. In the reconstructions, the axon is red and the soma and dendrites are black. (The scale bar is 10  $\mu\text{m}$  for all IR-DIC images, 100  $\mu\text{m}$  for the reconstructions in a–c, and 50  $\mu\text{m}$  for the reconstruction in d.)

PCRs for Cx36 and the other Cx due to considerable sequence divergence. For Cx36, specific primers were placed on the first and second coding exons. For the other Cx two independent analyses were performed. Common primers in the single coding exon served to amplify a composite DNA product, which was analyzed by Southern blotting with specific oligonucleotide probes for the presence of individual Cx sequences. To determine which of these sequences originated from reverse-transcribed RNA or from genomic DNA, PCR was performed on separate sets of cells with Cx subunit-specific primers placed to span the first intron.

In this manner, we obtained valuable information on Cx expression in identified neuronal cell types (Table 1). We found that all three neocortical cell types express Cx36. Moreover, a few visual cortex layer 2/3 bipolar interneurons also express Cx32. A representative PCR analysis is shown in Fig. 4b. In

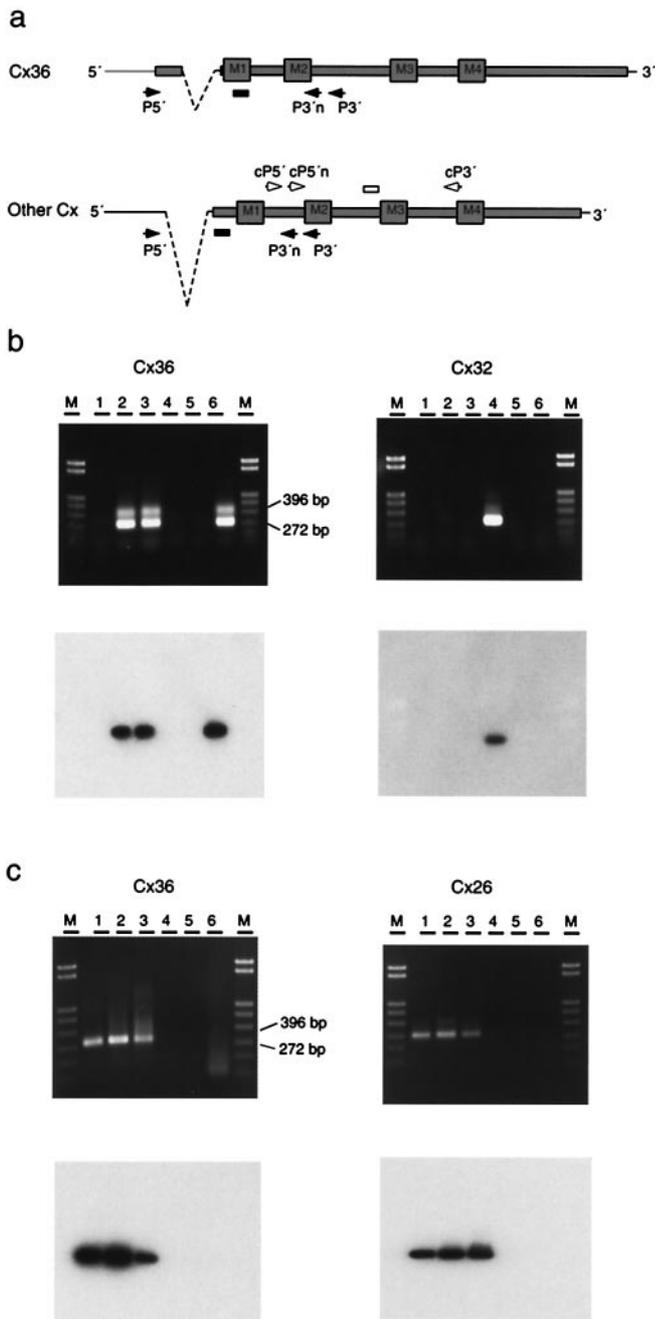


**Fig. 3.** Biochemical characterization of electrically coupled neurons. (a1) Biocytin-labeled bipolar interneuron in the visual cortex. (a2) *In situ* hybridization of the same section with a digoxigenin-labeled somatostatin riboprobe. (b1) Biocytin-labeled fusiform interneuron in the barrel cortex. (b2) *In situ* hybridization of the same section with a digoxigenin-labeled somatostatin riboprobe. (c1) Biocytin-labeled hippocampal dentate gyrus basket cell. (c2) Immunocytochemistry of the same section with a parvalbumin-specific antibody. (Scale bar, 20  $\mu\text{m}$ .)

hippocampal basket cells, Cx36 was found to be expressed in 8 of 21 and Cx26 in 6 of 21 cells. Other Cx could not be detected. Fig. 4c illustrates a representative PCR analysis from this cell type. The fact that fewer bipolar interneurons express Cx32 and that fewer basket cells express Cx26 compared with Cx36 could be due either to nonuniform expression or to differential sensitivity of detection. Moreover, Cx26 expression was found with specific primers but not with common primers in basket cells (see Table 1).

## Discussion

In this study we provide functional and molecular evidence for the presence of gap junctions in identified pairs of neurons. The main findings are as follows: (i) selective subsets of neurons in the cortex and hippocampus are electrically coupled well beyond early developmental stages in the rat brain. (ii) Electrical coupling occurs between cells of the same and different types.



**Fig. 4.** Identification of Cx expression by single-cell RT-PCR. (a) Location of PCR primers (arrows) and hybridization probes (bars) for the analysis of Cx expression in the cortical and hippocampal cell types. M1 to M4 denote the putative transmembrane regions. For Cx36 amplification the 5' primer (P5') was located on exon 1; the 3' primer (P3') and nested 3' primer (P3'n) were on exon 2. The expression analysis of the other Cx was a two-step procedure. First, generic Cx primers (white arrows) located on conserved coding region (exon 2) were used, and the constituents in the RT-PCR product were evaluated by Southern analysis, using subunit-specific probes (white bar) and direct DNA sequencing. Because the common, generic primers did not span an intron, the origin of the PCR amplicon was verified in separate reactions by use of Cx subunit-specific primers (black arrows), with the 5' primer located in the 5' untranslated region (exon 1) and the 3' and nested 3' primers located in the coding region (exon 2). The identity of the PCR products obtained with the specific primers was also confirmed by Southern analysis with Cx-specific oligonucleotide probes (black bar) and direct DNA sequencing. (b) Representative experiment from bipolar neurons in P14 rat visual cortex. Gel electrophoresis (Upper) was carried out for RT-PCR products, as was a corresponding Southern blot (Lower) for six bipolar neurons after amplification with specific

(iii) Cx36, Cx32, and Cx26 are differentially expressed in the cell types analyzed here.

These findings raise several unresolved issues. First, does electrical coupling exist in these cells beyond the age analyzed here? The electrical coupling between pairs of bipolar layer 2/3 neurons in the visual cortex demonstrates the existence of gap junctions in a structure that at the time of our analysis has not reached maturity. However, electrical coupling in more mature structures is demonstrated by its occurrence in the somatosensory cortex at P12–P18. Indirect evidence for the presence of electrical coupling in the adult brain comes from studies demonstrating that the major Cx subunit, Cx36, that we identified in the cell types analyzed here is expressed in the adult in a number of brain structures, including hippocampus and forebrain (5).

Second, further identification of electrically coupled neurons and their Cx is warranted. Our study provides evidence that gap junctions exist both between neurons of the same type (two GABAergic interneurons) and between neurons representing different cell types (a glutamatergic and a GABAergic neuron). Electrical coupling between two inhibitory interneurons was also found in two recent studies (13, 14). Our data corroborate this finding and show in addition that electrical coupling can even occur between an inhibitory and an excitatory neuron. Although the incidence of coupling between the fusiform GABAergic interneuron and the glutamatergic spiny stellate neuron was lower compared with that between interneurons of the same type, the example shown here is not unique. In an ongoing study we found electrical coupling between parvalbumin-positive fast-spiking multipolar interneurons and pyramidal neurons in layer 2/3 of the visual cortex (data not shown). For these pairs, too, the incidence of coupling is lower compared with that between the interneurons analyzed in this study. Furthermore, the widespread expression of Cx36 in the brain and its occurrence in other principal neurons (5) clearly indicate that electrical coupling between yet other types of neurons must occur. The *in situ* hybridization data of the Cx36 expression also clearly document its presence in CA3 pyramidal neurons, which could explain the previously reported electrotonic coupling between CA3 pyramidal cells (42).

Third, the molecular signature of Cx expression in these cell types reveals an intriguing complexity. In the electrically coupled pairs of neurons in the somatosensory cortex (the somatostatin-positive fusiform interneurons and the spiny stellate cells) only Cx36 expression was found. In two other types of neurons, on the other hand, expression of different Cx could be detected: Cx36 and Cx32 in the somatostatin-positive cells of visual cortex and Cx36 and Cx26 in hippocampal basket cells.

The differential expression of Cx leads to yet another important unresolved issue: the cellular localization of neuronal Cx. Anatomical studies (37) and modeling data (43) have suggested their presence on both dendrites and axons. Indeed, localization of gap junction channels far from the soma along with low expression could explain the scarcity of dye coupling observed in this study (data not shown) and elsewhere (14). Furthermore, it will be interesting to analyze whether in neurons expressing more

Cx36 and Cx32 primers, respectively. An RT-PCR product was obtained for three of six neurons with Cx36 primers and for one of another set of six cells with Cx32 primers. The upper band seen after amplification with Cx36 primers has been verified by sequencing to represent a single strand that runs at a height different from that of a double strand. (c) Representative experiment from basket cells of P14 rat hippocampus. Gel electrophoresis was carried out for RT-PCR products, as was a corresponding Southern blot for six basket cells after amplification with Cx36 and Cx26 primers, respectively. An RT-PCR product was obtained in three of six neurons with both primer pairs. M, size marker.

**Table 1. Connexin expression in identified neurons**

Connexin	Bipolar interneurons		Fusiform interneurons		Stellate cells		Basket cells	
	Common primers	Specific primers	Common primers	Specific primers	Common primers	Specific primers	Common primers	Specific primers
Cx36	ND	13/28	ND	20/42	ND	7/28	ND	8/21
Cx26	7/24	0/21	0/23	0/17	0/23	1/20	0/23	6/21
Cx32	5/24	4/24	0/23	0/17	0/23	0/20	0/23	0/21
Cx33	4/24	ND	0/23	ND	0/23	ND	0/23	ND
Cx43	5/24	0/19	3/23	0/21	0/23	ND	0/23	ND

In the visual cortex layer, two-thirds of the bipolar interneurons Cx36 could be amplified in 13 of 28 cells. After amplification with generic Cx primers, Cx26, Cx32, Cx33, and Cx43 were detected in the compound PCR product. Of these, only Cx32 (4 of 24 cells) but not Cx26 and Cx43 could be amplified with intron-spanning subunit-specific primers. Cx33-specific primers were not used, because previous work had indicated that this Cx is not expressed in the brain. These results indicate that amplification of Cx26, Cx33, and Cx43 with common primers was from genomic DNA. In somatosensory cortex layer four, an RT-PCR product with Cx36-specific primers was obtained in 20 of 42 fusiform interneurons and in 7 of 28 stellate cells. For the fusiform interneurons, the amplification of Cx43 with common primers was deduced to be of genomic origin, because none of 21 fusiform interneurons showed Cx43 expression when intron-spanning specific Cx primers were used. In basket cells of the hippocampus, Cx36 was detected in 8 of 21 neurons, and Cx26 was found in 6 of 21 neurons. ND, not determined.

than one type of Cx, gap junction channels may localize differently in the same neuron and may subservise distinct functions.

Finally, the extent of electrical coupling and thus the size of these networks in different brain regions remain to be determined and cannot be addressed with the methods used here. Thus, the higher rate of electrical coupling between nearby basket cells does not necessarily predict large networks if this coupling is restricted to “microdomains” (e.g., microcolumns in the cortex). On the other hand, large interconnected networks may occur if on average each neuron contacts more than one other neuron in local randomly connected networks (43).

Addressing these issues will help to define the role of gap junctions at both the cellular and the system level. Undoubtedly, the functional significance of gap junctions in the juvenile and the adult differs from that at early developmental stages.

The authors thank Ulla Amtmann for her help with the single-cell PCR experiments, Joachim Lübke and Roger Traub for helpful discussions, and Peter Seeburg for his help and advice throughout the course of the experiments and for critically reading the manuscript. This work was supported in part by the Schilling Foundation (H.M.) and by the Graduate Program of Molecular and Cellular Neurobiology of the University of Heidelberg (M.B.).

- Bennett, M. V., Barrio, L. C., Bargiello, T. A., Spray, D. C., Hertzberg, E. & Saez, J. C. (1991) *Neuron* **6**, 305–320.
- Dermietzel, R. & Spray, D. C. (1993) *Trends Neurosci.* **16**, 186–192.
- Bruzzone, R., White, T. W. & Paul, D. L. (1996) *Eur. J. Biochem.* **238**, 1–27.
- Dermietzel, R., Traub, O., Hwang, T. K., Beyer, E., Bennett, M. V., Spray, D. C. & Willecke, K. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 10148–10152.
- Condorelli, D. F., Parenti, R., Spinella, F., Trovato Salinaro, A., Belluardo, N., Cardile, V. & Cicirata, F. (1998) *Eur. J. Neurosci.* **10**, 1202–1208.
- Sohl, G., Degen, J., Teubner, B. & Willecke, K. (1998) *FEBS Lett.* **428**, 27–31.
- Lo Turco, J. J. & Kriegstein, A. R. (1991) *Science* **252**, 563–566.
- Yuste, R., Peinado, A. & Katz, L. C. (1992) *Science* **257**, 665–669.
- Yuste, R., Nelson, D. A., Rubin, W. W. & Katz, L. C. (1995) *Neuron* **14**, 7–17.
- Peinado, A., Yuste, R. & Katz, L. C. (1993) *Neuron* **10**, 103–114.
- Nadarajah, B., Jones, A. M., Evans, W. H. & Parnavelas, J. G. (1997) *J. Neurosci.* **17**, 3096–3111.
- Bennett, M. V. L. (2000) *Nat. Neurosci.* **3**, 7–9.
- Galarreta, M. & Hestrin, S. (1999) *Nature (London)* **402**, 72–75.
- Gibson, J. R., Beierlein, M. & Connors, B. W. (1999) *Nature (London)* **402**, 75–79.
- Christie, M. J., Williams, J. T. & North, R. A. (1989) *J. Neurosci.* **9**, 3584–3589.
- Michelson, H. B. & Wong, R. K. (1994) *J. Physiol. (London)* **477**, 35–45.
- Strata, F., Atzori, M., Molnar, M., Ugolini, G., Tempia, F. & Cherubini, E. (1997) *J. Neurosci.* **17**, 1435–1446.
- Mann-Metzer, P. & Yarom, Y. (1999) *J. Neurosci.* **19**, 3298–3306.
- Draguhn, A., Traub, R. D., Schmitz, D. & Jefferys, J. G. (1998) *Nature (London)* **394**, 189–192.
- Buzsaki, G., Horvath, Z., Urioste, R., Hetke, J. & Wise, K. (1992) *Science* **256**, 1025–1027.
- Ylinen, A., Bragin, A., Nadasdy, Z., Jando, G., Szabo, I., Sik, A. & Buzsaki, G. (1995) *J. Neurosci.* **15**, 30–46.
- Chrobak, J. J. & Buzsaki, G. (1996) *J. Neurosci.* **16**, 3056–3066.
- Chang, Q., Gonzalez, M., Pinter, M. J. & Balice-Gordon, R. J. (1999) *J. Neurosci.* **19**, 10813–10828.
- Agmon, A. & Connors, B. W. (1991) *Neuroscience* **41**, 365–379.
- Markram, H., Lübke, J., Frotscher, M. & Sakmann, B. (1997) *Science* **275**, 213–215.
- Feldmeyer, D., Egger, V., Lübke, J. & Sakmann, B. (1999) *J. Physiol. (London)* **521**, 169–190.
- Dotd, H. U. & Zieglgansberger, W. (1990) *Brain Res.* **537**, 333–336.
- Stuart, G. J., Dotd, H. U. & Sakmann, B. (1993) *Pflügers Arch.* **423**, 511–518.
- Koh, D. S., Geiger, J. R., Jonas, P. & Sakmann, B. (1995) *J. Physiol. (London)* **485**, 383–402.
- Reyes, A., Lujan, R., Rozov, A., Burnashev, N., Somogyi, P. & Sakmann, B. (1998) *Nat. Neurosci.* **1**, 279–285.
- Lübke, J., Markram, H., Frotscher, M. & Sakmann, B. (1996) *J. Neurosci.* **16**, 3209–3218.
- Catania, M. V., Tolle, T. R. & Monyer, H. (1995) *J. Neurosci.* **15**, 7046–7061.
- Standaert, D. G., Landwehrmeyer, G. B., Kerner, J. A., Penney, J. B., Jr., & Young, A. B. (1996) *Brain Res. Mol. Brain Res.* **42**, 89–102.
- Lambolez, B., Audinat, E., Bochet, P., Crepel, F. & Rossier, J. (1992) *Neuron* **9**, 247–258.
- Geiger, J. R., Melcher, T., Koh, D. S., Sakmann, B., Seeburg, P. H., Jonas, P. & Monyer, H. (1995) *Neuron* **15**, 193–204.
- Monyer, H. & Jonas, P. (1995) in *Single-Channel Recording*, eds. Sakmann, B. & Neher, E. (Plenum, New York), pp. 357–373.
- Katsumaru, H., Kosaka, T., Heizmann, C. W. & Hama, K. (1988) *Exp. Brain Res.* **72**, 363–370.
- Kawaguchi, Y. & Kubota, Y. (1996) *J. Neurosci.* **16**, 2701–2715.
- Cauli, B., Audinat, E., Lambolez, B., Angulo, M. C., Ropert, N., Tsuzuki, K., Hestrin, S. & Rossier, J. (1997) *J. Neurosci.* **17**, 3894–3906.
- Woolsey, T. A., Dierker, M. L. & Wann, D. F. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2165–2169.
- Lübke, J., Egger, V., Sakmann, B. & Feldmeyer, D. (2000) *J. Neurosci.* **20**, 5300–5311.
- MacVicar, B. A. & Dudek, F. E. (1981) *Science* **213**, 782–785.
- Traub, R. D., Schmitz, D., Jefferys, J. G. & Draguhn, A. (1999) *Neuroscience* **92**, 407–426.